Bioengineering

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The Alpha Beta Rearrangement of the Asp-Gly Sequence
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The protein proopiomelanocortin (POMC) is a precursor several nerve peptide hormones, including MSHs, ACTH, CLIP, LPH, β-endorphin, and the Joining peptide (JP). The biological activity of POMC derived peptides have been well studied except for JP. To investigate the biological function of JP, we chemically synthesized JP by an Fmoc solid phase method. However, the yield of the synthesized JP was poor and a highly efficient α/β-rearrangement in the Asp-Gly sequence was observed. Therefore, we evaluated the rearrangement of the Asp-Gly sequence under several conditions to estimate the biological activity and the stability of the correct configuration of JP.
For this purpose, JP was chemically synthesized by ordinary Fmoc and Boc solid phase methods. After deprotection, JP was separated and identified by reverse-phase HPLC and MALDI-TOF/MS, respectively. The rearrangement of the Asp-Gly moiety was observed in the case of the Fmoc method but was not significant in the case of the Boc method.
To estimate the stability of the Asp-Gly moiety, JP was treated with several buffers in the pH range of 1-8. The α/β-rearrangement was gradually increased in a pH-dependent manner and was significantly observed under strongly acidic conditions. In addition, salt effects for the rearrangements were also estimated. The results will be discussed in this paper.

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Photo-Regulation of Small G Protein Normal and Oncogenic K-Ras using Photochromic Molecules
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Ras is one of the small G-proteins known as a molecular switch mediating cellular signalling. Switching ON state of Ras is induced by exchange of bound GDP for GTP and OFF state is by hydrolysis of GTP to GDP. Interestingly, the core nucleotide-binding motif of Ras is considerably conserved with the ATP driven motor proteins, myosin and kinesin. Therefore, it is believed that these nucleotide requiring proteins share common molecular mechanism utilizing nucleotide hydrolysis cycle. Previously, we have incorporated photochromic molecules, 4-phenylazophenyl maleimide (PAM), into the functional site of kinesin as a photo-switching nano device and succeeded to regulate kinesin ATPase activities reversibly upon visible light (VIS) and ultra-violet (UV) light irradiation. Therefore, it is expected that Ras can be also regulated using photochromic molecules.
In this study, we performed basic study to control the function of Ras using photochromic molecules upon VIS and UV light irradiations. We prepared normal and oncogenic Ras mutants which have a single cysteine at functional sites and modified with photochromic molecules of azobenzene and spiropyran derivatives stoichiometrically. The GTPase activities of PAM-Ras were reversibly altered upon VIS and UV light irradiations. In order to monitor the effect on GTPase kinetic pathway by the photoisomerization of PAM, we synthesized fluorescent GTP analogue, NBD-GTP. The Kinetic studies suggested that the initial binding step of NBD-GTP to Ras and the dissociation step of NBD-GDP from Ras were regulated by the photoisomerization of PAM.

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Harnessing the Dynamical Movement of OmpG Loops for Protein Sensing
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Oligomeric protein nanopores with rigid structures have been engineered for the purpose of sensing a wide range of analytes including small molecules and biological species such as proteins and DNA. We chose a monomeric β-barrel porin, OmpG, as the platform from which to derive the nanopore sensor. OmpG is decorated with several flexible loops that move dynamically to create a distinct gating pattern when ionic current passes through the pore. Biotin was chemically tethered to the most flexible one of these loops. The gating characteristic of the loop's movement in and out of the porin was substantially altered by analyte protein binding. The gating characteristics of the pore with bound targets were remarkably sensitive to molecular identity - even providing the ability to distinguish between homologues within an antibody mixture. A total of five gating parameters were analyzed for each analyte to create a unique fingerprint for each biotin binding protein. Our exploitation of gating noise as a molecular identifier may open new possibilities for more sophisticated sensor design while OmpG's monomeric structure greatly simplifies nanopore production.

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Multicolor Monomeric Near-Infrared Fluorescent Proteins
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The genetically encoded near-infrared fluorescent probes are preferable for non-invasive in vivo imaging. In the near-infrared spectral region (650-900 nm) mammalian tissues are relatively transparent to light because the combined absorption by hemoglobin and water is minimal.
Previously, we have developed five spectrally distinct fluorescent proteins, iRFP670, iRFP682, iRFP702, iRFP713 (aka iRFP) and iRFP720, from bacterial phytochromes. As a chromophore, iRFPs use a heme derivative, called biliverdin, abundant in mammalian cells. All iRFPs incorporate endogenous biliverdin efficiently and autocatalytically, do not require its exogenous supply and, therefore, can be used as easily as GFP-like proteins. iRFPs are dimers and can mainly serve for labeling of organelles and whole cells. iRFPs have enabled multicolor imaging of deep tissues in living animals.
Here we report a palette of monomeric iRFPs suitable for protein tagging, which also do not require external biliverdin. To engineer these proteins we first used rational design to monomerize the proteins and engineer spectral shifts. Then we applied directed molecular evolution with the high-throughput screening for selection of mutants, which incorporate biliverdin efficiently and specifically. As the result, we have engineered three spectrally different monomeric iRFPs, named miRFP670, miRFP703 and miRFP709. miRFPs are characterized by high effective brightness in mammalian cells, high pH stability and high photostability. We demonstrated that miRFPs perform well as fusion tags for cellular proteins.
The set of miRFPs should enable imaging of several tagged proteins in living mammals, and thus will be useful in cell and developmental biology and biomedicine. The developed molecular evolution approaches could be used for optimization of genetically encoded tools derived from other photoreceptors including flavoproteins and opsins.

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Design and Characterization of Force-Sensitive DNA Origami Components
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Scaffolded DNA origami is powerful design and fabrication tool for the creation of nanoscale objects via bottom up self-assembly. These objects have ~nm level geometric complexity and spatial accuracy, which is comparable to biological machinery. DNA origami has been used to create different a wide range of objects such as drug delivery containers or platforms to guide molecular robots. Current applications of DNA origami exploit the large stiffness of bundles of dsDNA to create structures that maintain a well-defined and static geometry. However, DNA origami nanostructures with mechanically functional components, such as springs or actuators have remained largely unexplored. We aim to make DNA origami devices that are responsive to force magnitudes typically seen in biomolecular system (~picoNewtons). We have currently developed a binary approach to make force-sensitive DNA origami components and demonstrated this approach through the design of a binary force sensor. This force sensor incorporates structures similar to DNA hairpins into DNA origami designs. The hairpin-like structures undergo a conformational change at a specific force threshold. We have characterized the conformational change dynamics of this force sensor using different experimental methods including single-molecule total internal reflection fluorescence microscopy, transmission electron microscopy and magnetic tweezers. We have shown that such dynamics can be tuned according to the design to meet the requirement of a wide range of applications. An analog force sensor is also in development using similar approaches. Ultimately we aim to use these devices to measure forces of molecular interactions in cellular systems, for example cellular traction forces applied during cell migration.

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Remodeling Protein Interfaces to Regulate Recognition
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Protein affinity reagents play an important role across a wide-range of life science applications. Efforts to enhance affinity reagents through protein